

**What is Claimed is:**

1. An isolated polynucleotide which encodes a *Bacillus thuringiensis* insecticidal toxin or insecticidal fragment thereof, wherein said polynucleotide hybridizes under conditions selected from the group consisting of stringent hybridization conditions and specific hybridization conditions with one or more of the nucleotide sequences selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3 (tic901), SEQ ID NO:5(tic1201), SEQ ID NO:7 (tic407), SEQ ID NO:9 (tic417), SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, and SEQ ID NO:32 or with the complement thereof.
2. The isolated polynucleotide of claim 1 wherein said toxin is active against a coleopteran insect pest.
3. The isolated polynucleotide according to claim 2 wherein said coleopteran insect pest is selected from the group consisting of a corn rootworm and a Colorado potato beetle.
4. The polynucleotide according to claim 3 wherein said corn rootworm is selected from the group consisting of a western corn rootworm, a southern corn rootworm, or a northern corn rootworm.
5. The polynucleotide according to claim 1 wherein said nucleotide sequence is SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, and SEQ ID NO:32.
6. A polynucleotide comprising a nucleotide sequence which encodes an approximately 34 to about 39 kDa toxin active against a coleopteran pest, wherein said nucleotide sequence has been optimized for expression in plants, and wherein said toxin comprises the amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:31, and SEQ ID NO:33.
7. A host cell transformed to contain a polynucleotide encoding an insecticidal protein or insecticidal fragment thereof wherein said polynucleotide comprises a nucleotide sequence as set forth in a sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:13, SEQ ID NO:30, and SEQ ID NO:32.
8. The host cell of claim 7 wherein said host cell is a plant cell.

9. A recombinant bacterium comprising an isolated polynucleotide that encodes an insecticidal protein wherein said protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:31, and SEQ ID NO:33.

10. A method for controlling a coleopteran insect pest comprising contacting said pest with a pesticidal amount of an approximately 34 to about 39 kDa *Bacillus thuringiensis* toxin protein or insecticidal fragment thereof, wherein said toxin protein is selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:31, and SEQ ID NO:33.

11. A method for controlling a coleopteran insect pest comprising contacting said pest with a pesticidal amount of an approximately 34 to about 39 kDa *Bacillus thuringiensis* toxin protein or insecticidal fragment thereof, wherein said toxin protein is encoded by a nucleotide sequence that is or that hybridizes under stringent conditions to a nucleotide sequence comprising at least 18 consecutive nucleotides selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:30, and SEQ ID NO:32.

12. An isolated insecticidal protein derived from *Bacillus thuringiensis* that is from about 34 kDa to about 39 kDa and is encoded by a nucleotide sequence that is or that hybridizes under stringent conditions to a nucleotide sequence comprising at least 18 consecutive nucleotides selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:30, and SEQ ID NO:32.

13. An isolated insecticidal protein derived from *Bacillus thuringiensis* that is from about 34 kDa to about 39 kDa and is encoded by a nucleotide sequence that is or hybridizes under stringent conditions to at least 18 consecutive nucleotides selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, and SEQ ID NO:32, and wherein said *Bacillus thuringiensis* is selected from the group consisting of EG2158, EG6489, EG6561, EG3618, EG6555, EG6618, 86833, and EG4653.

14. A method for detecting a first nucleotide sequence encoding an insecticidal protein wherein said first nucleotide sequence hybridizes to a second nucleotide sequence that is selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3 (tic901), SEQ ID NO:5(tic1201), SEQ ID NO:7 (tic407), SEQ ID NO:9 (tic417), SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, and SEQ ID NO:32 (tic431) or with the complement thereof under stringent hybridization conditions.

15. A nucleotide sequence that encodes an insecticidal protein or insecticidal fragment or homolog thereof, wherein said insecticidal protein or insecticidal fragment or homolog thereof is selected from the group of sequences consisting of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:31, and SEQ ID NO:33.

16. A method for detecting a first nucleotide sequence encoding an insecticidal protein, comprising the steps of (a) producing an amplicon from a template comprising said first nucleotide sequence in a sample using amplification primers comprising the sequences as set forth in SEQ ID NO:13 and SEQ ID NO:14; (b) isolating and purifying the amplicon; (c) isolating and purifying the complete first nucleotide sequence encoding said insecticidal protein based on the isolated and purified amplicon sequence; and (d) expressing said insecticidal protein in a host cell from said first nucleotide sequence, wherein said first nucleotide sequence hybridizes to a second nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:30, and SEQ ID NO:32 or complements thereof under stringent hybridization conditions.

17. The method according to claim 16 wherein said host cell is selected from the group of cells comprising a plant cell, a bacterial cell, a fungal cell, an insect cell, and a mammalian cell.

18. The method according to claim 17 wherein said host cell is a plant cell selected from the group of plant cells comprising a monocot plant cell and a dicot plant cell.

19. The method according to claim 18 wherein said monocot plant cell is selected from the group of plant cells comprising a corn plant cell, a wheat plant cell, a rice plant cell, an oat plant cell, an onion plant cell, and a grass plant cell.

20. The method according to claim 18 wherein said dicot plant cell is selected from the group of plant cells comprising a cotton plant cell, a canola plant cell, a soybean plant cell, a fruit tree plant cell, an okra plant cell, a pepper plant cell, an ornamental plant cell, a sunflower plant cell, a cucurbit plant cell, and a melon plant cell.

21. An isolated nucleic acid molecule comprising a polynucleotide sequence encoding a toxin protein wherein said toxin protein comprises a sequence that exhibits at least about 70% sequence identity to a nucleotide sequence selected from the group of amino acid sequences consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:31, and SEQ ID NO:33, or a coleopteran-active variant or portion thereof.

22. An isolated nucleic acid molecule comprising a polynucleotide sequence which encodes a protein toxic to coleopteran insect pests, wherein said polynucleotide sequence hybridizes to a nucleic acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3 (tic901), SEQ ID NO:5(tic1201), SEQ ID NO:7 (tic407), SEQ ID NO:9 (tic417), SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, and SEQ ID NO:32 (tic431), or with the complement thereof under stringent hybridization conditions.

23. The isolated nucleic acid of claim 1 or 5 wherein said polynucleotide sequence is isolated from a bacterium.

24. The isolated nucleic acid of claim 6 wherein said polynucleotide sequence is isolated from *Bacillus thuringiensis*.

25. A substantially purified toxin protein wherein said toxin protein comprises a sequence that has at least about 70% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:31, and SEQ ID NO:33.

26. The substantially purified toxin protein of claim 25, wherein said toxin protein comprises SEQ ID NO: 4.

27. A recombinant DNA construct, comprising a polynucleotide sequence encoding an insecticidal protein, wherein said polynucleotide sequence is at least about 70% identical to SEQ ID NO:13, said insecticidal protein being selected from the group consisting of all or an

insecticidal fragment of a protein as set forth in SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:31, and SEQ ID NO:33.

28. The recombinant DNA construct of claim 27, wherein said polynucleotide sequence is as set forth in SEQ ID NO:3.

29. The recombinant DNA construct of claim 28 further comprising a promoter or partial promoter region operably linked to said polynucleotide sequence.

30. The recombinant DNA construct of claim 29 wherein said promoter is a constitutive promoter, an inducible promoter, or a tissue-specific promoter.

31. The recombinant DNA construct of claim 30, wherein the promoter is a cauliflower mosaic virus (CaMV) 35S promoter.

32. The recombinant DNA construct of claim 31 further comprising an intron.

33. The recombinant DNA construct of claim 32 wherein said intron is an hsp70 intron.

34. The recombinant DNA construct of claim 33 further comprising an operably linked 3' non-translated region.

35. A recombinant host cell transformed with a polynucleotide sequence of claim 1 or claim 27 encoding an insecticidal protein, wherein said recombinant host cell expresses said protein that is at least about 70% similar in amino acid sequence to an insecticidal protein selected from the group consisting of SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:31, and SEQ ID NO:33.

36. The recombinant host cell of claim 35, wherein said recombinant host cell is a plant cell.

37. A method for expressing an insecticidal protein in a plant cell, comprising the steps of:

- (a) inserting into the genome of a plant cell a nucleic acid sequence comprising:
  - (i) a promoter that functions in said plant cell operably linked to,
  - (ii) a structural DNA encoding a protein selected from the group consisting of SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:31, SEQ ID NO:33, and insecticidal proteins that are at least about 70% similar to said group of proteins, and
  - (iii) a 3' non-translated DNA sequence that functions in the plant cell to cause transcription termination and polyadenylation;
- (b) obtaining said plant cell transformed to contain the nucleic acid sequence of step (a); and
  - (c) regenerating from the transformed plant cell a genetically transformed plant that expresses an insecticidal amount of said insecticidal protein.

38. A seed produced from the transformed plant of claim 37.

39. A plant grown from the seed of claim 38.

40. A biological sample derived from a plant, tissue, or seed, wherein said sample comprises a nucleotide sequence encoding an insecticidal protein selected from the group consisting of SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:31, and SEQ ID NO:33.
41. The biological sample of claim 40 comprising plant, tissue, or seed of a transgenic plant encoding said protein.
42. The biological sample of claim 41 wherein said sample is selected from the group consisting of an extract obtainable from the transgenic plant, and wherein said extract comprises a nucleotide sequence encoding an insecticidal fragment of a protein selected from the group consisting of SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:31, and SEQ ID NO:33.
43. The biological sample of claim 42 wherein said sample is selected from the group consisting of corn flour, corn meal, corn syrup, corn oil, corn starch, and cereals manufactured in whole or in part to contain corn by-products.
44. An extract derived from a transgenic corn plant, tissue, or seed comprising a nucleotide sequence encoding an insecticidal fragment of a protein selected from the group consisting of SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:31, and SEQ ID NO:33.
45. The extract of claim 44 wherein said sequence is detectable in said extract using a nucleic acid amplification or nucleic acid hybridization method.
46. The extract of claim 45 comprising plant, tissue, or seed of a transgenic corn plant expressing said protein.
47. The extract of claim 46 wherein said sample is selected from the group consisting of corn flour, corn meal, corn syrup, corn oil, corn starch, and cereals manufactured in whole or in part to contain corn by-products.

**Figure 1A**

161	T G S T Q K M K T D S F Q R D Y T K S E S T S V T N G L Q L G F K V A K G V V	200
161	T G T T Q K M K T D S F Q R D Y T K S E S T S V T N G L Q L G F K V A A K G V V	
161	M G T T P Q K M K S D S F Q R D Y T K S O S T S V T N G L Q L G V K V S A K G T V	TIC901
161	T G T T Q K M K T D S F Q R D Y T K S O S T S I T D G L Q L G F K V S A K G I V	TIC1201
161	T G T T Q K M K T D S F Q R D Y T K S Q S T S I T N G L Q L G F K V S A K G I V	TIC407
161	T G T T Q K M K T D S F Q R D Y T K S Q S T S V T N G L Q L G F K V S A K G V I	TIC417
161	T G T T Q K M K T D S F Q R D Y T K S Q S T S V T N G L Q L G F K V S A K G V V	TIC431p
	+	Consensus
201	A L A G A D F E T S V T Y N L S T T T E T N T I S D K F T V P S Q E V T L P P	240
201	A L A G A D F E T S V T Y N L S S T T A T E T D T I S D K F T V P S Q E V T L S P	
201	V L G E A S S L E T S V T Y N L S S T T A T E T D T I S D K F T V P S Q E V T L P P	TIC901
201	A L A G A D F E A S V N Y N L S T T A T E T N T I S D K F T V P S Q E V T L A P	TIC1201
201	A L A G A D F E A S V N Y N L S T T A T E T N T I S D K F T V P S Q E V T L A P	TIC407
201	A L A G A D F E T S V T Y N L S T T A T E T N T I S D K F T V P S Q E V T L P P	TIC417
	+	TIC431p
241	G H K A I V K H D L R K M V Y S G T H D L K G D L I V S F N D K E I V Q K F I Y	280
241	G H K A V V K H D L R K M V Y S G T H D L K G D L K V G F N D K E I V Q K F I Y	
241	G H K A V I K H D L R K M V Y S G T H D L K G D L K V A F N D K E I V Q K F I Y	TIC901
241	G H K A I V K H S L K K M V Y S G T H D L K G D L T I T F N D K D L V Q K F I Y	TIC1201
241	G H K A I V K H S L K K M V Y S G T H D L K G D L T I T F N D K D L V Q K F I Y	TIC407
241	G H K A I V K H D L R K M V Y S G T H D L K G D L K V X F N D K E I V Q K F I Y	TIC417
241	G H K A I V K H D L R K M V Y S G T H D L K G D L K V X F N D K E I V Q K F I Y	TIC431p
	+	Consensus
281	P N Y R E I N N S D I R E T M I E I D E W N H V N P V N F Y E L V G V K N H ' K	320
281	P N Y R S I D L S D I R K T M I E I D K W N H V N T I D F Y Q L V G V K N H I K	
281	P N Y R S I D L S D I R K T M K E I D E W N H V K P I D F Y Q L V G I K N H I K	TIC901
281	P N Y K A I D L S N I R K A M T E I D E W N H V K P I D F Y Q L V G N K N Y I K	TIC1201
281	P N Y K A I D L S N I R K A L T E I D E W N H V K P T D F Y Q L V G N K N Y I K	TIC407
281	P N Y R S I D L S D I R K T M I E I D E W N H V N P I D F Y Q L V G V K N H I K	TIC417
281	P N Y R S I D L S D I R K T M I E I D E W N H V N P I D F Y Q L V G V K N H I K	TIC431p
	+	Consensus

Figure 1B

<pre> 321 N G E T L Y I D T P A K F M F N G A N P Y Y R A T E F T E Y D G N N N P V Q T - K 321 N G D T L Y I D T P A E F T F N G A N P Y Y R A T F T E Y D E N G N P V Q T - K 321 N G D T L Y I E T P A K F I F N G A N V Y Y R A T F T E Y D K D G K P V Q F N K 321 N G D T L Y I E T P A K F T L N G G N P Y Y T A T F T E Y D E N G N Q V K T - K 321 N G D T L Y I E T P A K F T L N G G N P Y Y T A T F T E Y D E S G N Q V K T - K 321 N G D T L Y I D T P A K F T F N G A N P Y Y R A T F T E Y D E N G N P V Q T - K </pre>	<pre> TIC901 TIC1201 TIC407 TIC417 TIC431p Consensus </pre>
<pre> 330 340 350 </pre>	<pre> 330 340 350 </pre>
<pre> 360 V L S E N F K L 367 360 I L S G N 364 361 F L S E N Y K L 368 360 R L - N N - K 364 360 H L S V - - K 364 X L S E N - K L </pre>	<pre> TIC901 p TIC1201p TIC407p TIC417p TIC431p Consensus </pre>

Figure 1C